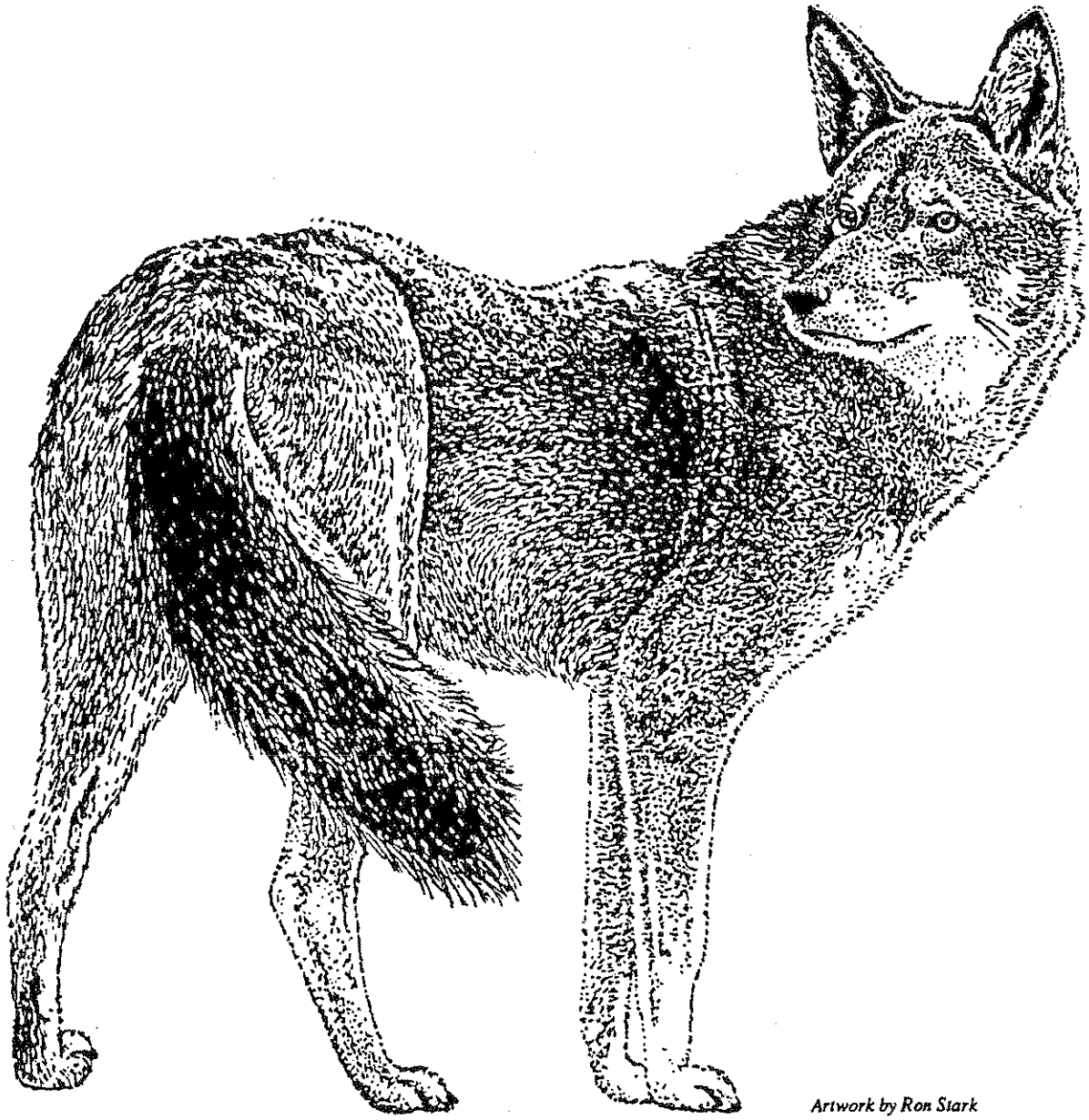
APPENDIX F

Red Wolf Husbandry Manual

Red Wolf Husbandry Manual



Artwork by Ron Stark

RED WOLF HUSBANDRY MANUAL

GUIDELINES FOR CAPTIVE MANAGEMENT

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Acknowledgements

Much of the information contained in this document was first compiled by Mr. Curtis J. Carley, the first red wolf project leader with the U. S. Fish and Wildlife Service, in a draft document entitled Guidelines for the Management and Husbandry of Captive Red Wolves being Raised for Reestablishment in the Wild. The following summarizes a recurrent theme from that document: "It is hoped that the reading of these guidelines will instill a feeling for the overall intent of the program. Should unforeseen events occur, those concerned should not hesitate in seeking further guidance from program advisors. If the program is to succeed, it is imperative that all involved facilities and personnel maintain open channels of communication."

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Red Wolf SSP©
Guidelines for Captive Management

INTRODUCTION

The partnership between the red wolf SSP (RWSSP) and the U. S. Fish and Wildlife Service (Service) has been an important part in implementing strategies outlined for the recovery program. Considered extinct in the wild in 1980, the red wolf's survival depended a great deal on the commitment and cooperation of the zoo community.

The red wolf was approved for American Zoo and Aquarium Association Species Survival Plan designation in 1984. At that time there were four facilities outside Point Defiance Zoo & Aquarium (PDZA) which housed red wolves and a total population of 57 animals, all of which were managed within the captive program. Recognition that the RWSSP was a key component in developing a comprehensive approach for red wolf restoration led to the first Service recovery plan that integrated captive management as a strategy for species conservation.

As of June 1, 1998, 41 different facilities were involved in red wolf captive management. Thirty-four involve RWSSP cooperators. PDZA operates two facilities, one at the zoo and another at an off-site compound. The Service operates two facilities associated with the mainland reintroduction projects. Three island propagation projects currently support red wolf population management and reintroduction. The Service manages two of the islands and one is managed by the state of Florida.

The total (wild and captive) red wolf population as of June 1, 1998 ranged from 140.118.0 (258) to 170.148.16 (334). This range reflects free-ranging wolves that are considered fate unknown in which radio contact has been lost and wild-born wolves not yet captured. Of the total population, 103.95 (198) were managed in captivity, including wolves at mainland reintroduction captive facilities and all wolves occurring at three island propagation sites.

From a conservation biology perspective, the purpose of the RWSSP is to maintain the genetic and demographic health of the population. From a species-specific standpoint, the objectives are to propagate and manage red wolves that are representative of their species and behaviorally suitable for potential reestablishment in the wild. The challenge to the RWSSP is to combine these two strategies and focus on minimizing, to the extent possible, adaptation to the captive environment, specifically human tolerance. Personnel working with red wolves (especially those on a day to day basis) must not attempt to modify the

animals' behavior. Avoidance of socialization or familiarization of the wolves with humans is fundamental to these guidelines and is a philosophy that must be maintained in every aspect of captive red wolf husbandry.

As the number of free-ranging red wolves increases, whether through reintroduction or propagation in the wild, it becomes increasingly difficult to identify which red wolves will be selected for release. However, attention to these guidelines should benefit facilities housing red wolves that may never be used for release. The advantage will be in the form of animals whose actions and responses are perhaps more predictable, which should make capture, handling, transport, and medical examinations less stressful to the wolves.

No protocol can cover all situations that might arise and these guidelines may need to be tailored according to facility location, enclosure design, individual animals, etc. Unusual events should be managed on a case-by-case basis in consultation with the RWSSP Coordinator and program advisors.

Use of trade names in this document does not imply endorsement by PDZA or the Service. Such trade names simply indicate that these products are acceptable in quality and function. Other products of equal quality or function may also be acceptable.

SECTION 1

Enclosure Requirements

1.1 Introduction. Red wolves can generally be described as shy and should receive as much privacy and little disturbance as possible. Pens are designed to prevent the wolves from escaping and to provide security from intruders, while promoting natural activities within a captive setting. Pens should be designed in an area that drains well to prevent the collection of water in the yard, especially in the vicinity of the dens. Many wolves use elevated areas (berms, top of den box) for resting which can promote better visibility to visitors. Soil, grass, tree and shrub plantings (for hiding and shade), hollow logs and similar materials can be included in the design to promote natural activities for the wolves. Digging in and around log and rock outcroppings should be monitored so the wolves do not dig extensive holes that would make retrieval of adults or pups difficult. Cooperators are encouraged to plan the construction of more than one pen at their facility to allow flexibility in management. However, this is not a prerequisite for approval to participate in the RWSSP. Isolation from other canids or conspecifics is not a requirement for successful management of red wolves.

1.2 Containment Barriers. Red wolves are generally easy to maintain, but can be hard on a pen through daily activity patterns. This can be compounded by pens that are too small, especially as pups get older or during periods of prolonged rain. Pen space requirement per each adult pair (with or without pups) should be no less than 5,000 sq. ft. (450 m^2) Fencing must be constructed of 9 gauge, 2 in. (5 cm) chainlink mesh suspended on 2 in. (5 cm) upright metal pipes that have been set in concrete. Vinyl coated wire is not recommended because the wolves can easily chew off and possibly swallow pieces of this material. The vertical height should be at least 8 ft. (2.4 m) with the addition of a 3 ft. (90 cm) wide overhang, of the same specifications as the vertical fence, that is slanted in at a 45 degree angle. Additional steps to reinforce the top of pen corners can be taken, as this is the likely spot that red wolves will jump when avoiding capture. Hot wire should not be necessary if guidelines are followed; however it should be noted that hot wire (when used) has been an effective measure of "insurance."

When pups are born, all sections of the fence should be checked, especially around gates. It may be necessary to add some sections of smaller dimension wire near the base of a gate on the hinge and lock sides to prevent pups from squeezing through.

For facilities with multiple pens, common fence lines must be avoided when using 2 in. (5 cm) mesh fencing. Past experience has taught us that both young and adult

wolves can sustain serious injuries when an appendage is stuck through the fence housing another wolf. When a fence line is shared, a strong 8-9 gauge 1 in. (2.5 cm) chainlink mesh must be used if resources permit. Heavy gauge, 1 in. x 1 in. (2.5 x 2.5 cm) hardware cloth, hog ringed to the existing fence can also be used. An alternative is to create at least an 18 in. (45 cm) buffer between the two pens. Gates, holding and shifting areas which may connect adjacent pens, and any gaps between the gate and the gate hinges, must incorporate similar design strategies.

Some red wolves may habitually dig at the base of the vertical fence. To prevent wolves from digging out, a 2-3 ft. (60-90 cm) wide section of chainlink fencing should be installed by lacing it with smooth wire or hog rings to the base of the vertical fencing. This digging barrier should come to the fence at an approximate right angle to the vertical fence and should be buried from 6-12 in. (15-30 cm) below ground level. A concrete footing approximately 6-8 in. (15-20 cm) wide, 3-4 ft. (90-120 cm) deep, should be poured at all gates to prevent digging at this area where a digging barrier cannot be installed. Fence lines must be inspected daily to detect any need for repairs, sharp protrusions, and to fill in substantial holes to prevent possible escape or injury.

1.3 Holding and Shifting Areas. Off exhibit holding and shifting areas for capture or quarantine can enhance management capabilities. However, these areas are not required for successful red wolf management. These areas should be easily accessible from the main enclosure and the wolves should be habituated to them through daily access. Similar fence specifications apply to these areas as used for common fence lines. In some cases visual separation may be desirable. Off exhibit holding or shifting areas, particularly when used for restraint or capture, should be fully covered and high enough, 10 ft. (3 m) to allow for flight/jump reaction of an animal.

1.4 Shelter. The wolves should have access to shade for warmer parts of the year. The design of a pen for red wolves can incorporate existing plantings into the design of the pen. Red wolves tolerate cold weather conditions but must be provided with a dry den structure, with dry bedding such as straw to be able to get out of rainy or windy conditions. Supplemental heating in the den box is generally not required.

(a) Den Boxes. Den boxes must be readily accessible by keepers, e.g. removable back or top, for cleaning and easy removal of wolves. This also allows accessibility for routine examinations, blood draws, inoculations, etc. Red wolf den boxes need not be spacious. The following measurements can be used as a guideline for den box construction, 4x5x4 ft. h. (90x150x190 cm) with a slight slope to the back of the den box, to allow for water runoff. A den entrance dimension of 18 x18 in. (45 x 45 cm) is sufficient and can be designed with a door that can be closed to prevent a wolf from exiting the

box. Two den structures should be available to the wolves. This will give the female a choice for whelping and the male an alternate source of shelter if the female occupies one den box with a litter of pups. A sufficient layer of dust free straw provides an effective bedding material that is easily cleaned.

(b) Natural Dens. Individual wolves will occasionally dig their own dens, which is normal behavior. This activity may be in response to seeking an alternate den selection for whelping, comfort/security, insect avoidance, etc. This activity should be closely monitored. Wolf-made dens can make the task of inspecting, removing, treating or monitoring adults and pups difficult and dangerous. There is also the possibility of the wolf-made den collapsing and creating a greater problem. The length, depth and location (under perimeter fence digging barrier) of the den and soil type are points cooperators should consider with regard to allowing or filling the den. Digging around the outside of the artificial den should be monitored for the reasons described above. Attaching and burying a digging barrier of chainlink to the skirt of the den can hinder this activity.

1.5 General Requirements. Professionally accepted zoological procedures are to be used on a routine basis. Pens should be checked daily to ensure that wolves cannot escape or injure themselves. Each wolf should be visually checked daily for general health. Food and water intake and general appearance and amount of stools should all be monitored and recorded in daily report forms, as patterns may indicate a problem with an animal.

(a) Daily Interactions. While the wolves are to be treated with respect, they should not be feared. If the animals' sense fear in their handlers they may begin "testing" certain handlers and may eventually become more aggressive and unmanageable. Keepers should have rake, shovel, etc., when entering the pen and maintain visual contact with the wolves while servicing the pen. In general, the wolves become ill at ease when intently stared at from within their confines. Should a wolf approach a keeper, whether out of curiosity or aggression, the wolf should be rebuffed with whatever tool is in hand while being sternly told "no". Under no circumstances should the keeper retreat, unless of course they feel their life is threatened. After a few tests the animal will usually give up such confrontations, however keepers should take care that such activities do not become a game for the animal. Alternatively, areas to shift the wolves into while the pen is being serviced can be included or added to the main enclosure.

(b) Sanitation. Hard-surface holding areas are to be cleaned daily and disinfected weekly unless directed otherwise by your veterinarian. Removal of fecal material from natural substrate should occur daily. Food containers must be cleaned and disinfected daily. Facilities with multiple red wolf pens

or other canids should use footbaths, filled with disinfectant, prior to entering the pen or holding area. All cleaning aids, disinfectants and chemical agents must be safe, nontoxic, and biodegradable.

(c) Water. Fresh, clean water for drinking must be available at all times. During the summer months it may be necessary to water twice a day. All watering containers must be cleaned and disinfected daily. Portable water containers should be sturdy 2 gal. (7.6 L) and ideally stainless steel. The containers can be clipped to the fence to prevent the wolves from tipping the container over. A small shallow pool can be planned into the display, which may be desirable in locations where summer temperatures are extreme. However, when pups are in the enclosure, the pool should be drained to an appropriate level or shallow water container used. All cleaning aids, disinfectants and chemical agents must be safe, nontoxic, and biodegradable.

SECTION 2

Identification and Records

2.1 Introduction. Complete records should be maintained on each animal and as a general rule, a wolf's studbook/identification number should be confirmed when handled.

2.2 Record Keeping. Health, medical, dietary, reproductive, and mortality records for each animal should be kept in accordance with the holding facilities record keeping system. Written daily reports should be maintained indicating significant events regarding the wolves' general condition, food consumption, bowel habits, animal interactions, etc. Copies of pertinent records should accompany each animal whenever it is transferred to another facility. Copies of the records should also be provided to the RWSSP coordinator upon request, or whenever the institution feels there is something significant to report. Reports on reproduction, mortalities, and transfers for the species should be made monthly to the International Species Information System (ISIS) Record Keeper. Births, deaths and transfers should also be reported promptly to the RWSSP coordinator.

2.3 Identification. The RWSSP has used the Trovan transponder as a permanent identification method for the red wolf population. Due to litigation involving Trovan, use of other microchip systems may be required in the future. It is important that whatever identification system is used, that the Trovan reader can read the microchip. The RWSSP coordinator will send, upon request, the transponders and implanter syringe to those facilities that do not have the equipment. All that is required is to borrow a reader from a nearby facility to read the transponder to insure that the chip has been properly installed. Transponder numbers should be sent to (or phoned to) Sue Behrns for inclusion in the SPARKS database and should accompany paper work (along with the studbook number) when a wolf is being transferred to another facility. It is not necessary to anesthetize the wolves to implant a microchip, however if the wolf is scheduled to be anesthetized for any reason, this is a good time to implant the chip. Wolf pups can be transponded in conjunction with the last scheduled vaccination. The area of implant is sub-cutaneous between the scapulas.

SECTION 3

Capture and Restraint

3.1 Introduction. It is difficult to characterize an individual wolf's response to capture with the possible exception that they are frightened. A wolf may respond by lying quietly without much struggle or act defensively by "snapping" at anything close to its mouth. To achieve a quick and successful capture of the animal, keepers familiar with the habits of the wolves are vitally important to this operation. Appropriate animal keepers, curatorial, and veterinary staff should coordinate, in advance, all captures in a well-planned manner.

Whenever touched by humans, the animal should be firmly but humanely handled. During handling it will experience such procedures as restraint, inoculations, blood drawings, etc. Keepers may understandably feel a personal need to stroke, pet, or scratch a wolf behind the ears, etc., when the animal is restrained. Such actions must be avoided. A clear understanding and periodic reinforcement of the red wolf program's objective of not modifying red wolf behavior or avoidance of socialization with people, and the potential problems associated with such actions should prevent such activity.

(a) Stress. Stress and heat are the two main concerns when capturing red wolves, therefore a capture should be coordinated with outdoor temperature in mind. The first indication that a red wolf is becoming overheated is generally the appearance of excessive panting and drooling or a white frothy foam around the mouth, reddened eyes and gums and in extreme cases vomiting. If the animal has become comatose or there is a fear that it soon will, cool immediately with water from a hose, drape with cool wet towels or submerge in cool water to aid in reducing its body temperature. Ice packs may be placed on the extremities or between the rear legs in the groin region. If symptoms of overheating occur and the wolf has not been captured, attempts at capture should cease immediately. The veterinarian and curator should then determine if further attempts at capture can resumed or be postponed until another time. Once captured and in the crate, the wolf should be placed in a shaded area or air-conditioned building. The crate should be no larger than is necessary for the animal to lay down in or turn around. The animal may be further calmed by loosely covering the cage with a light tarpaulin to restrict sights and sounds that may further excite the animal. If covered, the animal should be periodically monitored.

(b) Capture in a Confined Area. One of the quickest and least stressful ways of capturing a red wolf is to allow it to run into its den or other confined area (holding or shift area). This underscores the importance of coordination with

keepers familiar with the wolves' habits. Once the wolf has been observed running into its den, the entrance of the den should be blocked with a net or door incorporated into the den to prevent the animal from running out. At this point personnel familiar with the habits of the animal and knowledgeable with the use of a catchpole can access the wolf from the den entrance, from the top of a den with removable roof, or from a back door incorporated into the den. The type of catchpole recommended is a 5 ft. (150 cm) pole available from the Ketch-All Company. The ends of these particular snares have swivels, which help to prevent the noose from twisting down around the wolf's neck. The noose of the catchpole can be slipped over the head of the animal and secured. Most procedures (inoculations, blood draws, etc.) can normally be accomplished without removing the wolf from the den.

If the wolf is to be removed for transport, a crate should already be positioned, standing on end, outside the den as the animal is lifted or pulled from the den. The person holding the snare is the person in control and coordinates the operation. As the wolf is lifted or pulled from the den, another staff person should be available to assist the person controlling the snare by grabbing the wolf by the tail and/or hind legs. The wolf should never be grabbed above the base of the tail, as control of the head by no means insures that the wolf is not capable of turning sideways to bite. The keepers on each side of the den should not grab for the wolf until the person holding the catchpole indicates the wolf is under control. The wolf is then lifted in a coordinated manner for placement into a crate. When the animal's hindquarter is above the crate, that end is released as the person controlling the snare allows the weight of the wolf to drop into the crate. The snare is then released and removed from the wolf's head and the crate lowered to its proper position. An alternative method would be to place the crate door at the den door and attempt to run the wolf directly into the crate.

(c) Capture in an Open Area. Wolves that cannot be captured in a confined area may require capture with nets. This operation requires 3 to 4 people or more depending on the size of the enclosure. Facilities housing multiple wolves, e.g. adults with older pups, should give the wolves the opportunity to run into the den and then block their exit. Wolves that will not go into the den should be captured first to avoid prolonged running and possibly colliding with pen mates. Then place the wolves in a crate or other holding area and proceed with the denned animals until the work is completed. If the animal cannot be systematically worked into a corner of the pen for capture, it will be required to catch the animal as it runs the perimeter of the pen. Do not chase the animal; be in a position to stick your net in front of the animal as it runs by you. To avoid further stress and possible injury from excessive struggling, the animal's head should be quickly pinned to the ground or against the fence with the metal hoop part of a second net or V-stick by

another member of the catch team. The wolf can then be transferred directly into a crate, which has been brought to the point of capture, by one of four methods:

1. Positioning the crate along a fence line with the opening of the net abutted to the opening of the crate and forcing the wolf directly from the net into the crate. The crate can then be tipped up and the door closed.

2. Using a catch pole to pick the wolf up for placement in the crate, similar to the procedure for removing a wolf from a den box or "pushing" the animal directly into a crate that is in the horizontal position.

3. If the wolf is too tangled in the net, it may be required to grab the wolf firmly by the scruff of the neck by hand and then transfer the wolf into the crate. Essentially, the hands act as a catchpole, however this may be the least desirable method for obvious reasons.

4. In some cases a crate can be set in a corner of the pen and positioned in such a way that when the animal is forced to that corner and will seek refuge in the crate.

(d) Muzzles. For some procedures a wolf may require muzzling. To muzzle a wolf one handler should be in control of the head. With a strong 4 ft. (90 cm) piece of gauze (or soft cotton rope), the center point of the gauze is brought under the animal's chin at a line approximately between the nose and the eyes which is then tied with a half hitch or single knot over the top of the nose. The trailing ends are then brought under the animal's muzzle and behind the animal's head where it is tied in a bow for ease of removal. As a precaution, the jaws can be tied a second time. A nylon muzzle (wolf size) may be slipped over the gauze muzzle if needed or the nylon-type muzzle may be used alone. To remove the gauze and/or muzzle, pull one of the trailing ends of the bow and/or the loosened end of the muzzle and pull away, allowing the muzzle to slip away from the wolf's mouth.

3.2 Escape Procedures. There have been several reported escapes of red wolves from RWSSP facilities in the 20+ years that red wolves have been maintained in captivity. While it is difficult to determine the way in which an individual wolf will react to being outside its pen, implementing your facility's own escape procedures is the prudent first step. However, it is likely that these procedures will be general in nature to cover the variety of animals in each collection. The following procedures refer more specifically to red wolf escapes and should be followed to increase the chance of a quick and safe recovery of an escaped wolf.

1. Call the RWSSP coordinator immediately after assessing the situation and initiating the facility's own escape procedures. Every effort should be made to speak directly to the coordinator, regardless of the time of day, weekends, holidays, etc. If calls are made during normal business hours and you are given the option of leaving a message or staying on the line to speak to an operator; do both. Do not assume that the message will be retrieved immediately, so it is important to speak to the zoo operator. The coordinator should be called, even if the animal has already been captured, to review details of the escape, what did (or did not) work, etc. Such communications will be valuable to the RWSSP should other escapes occur.

2. When out of a pen, specific patterns that an escaped wolf will demonstrate may be extremely variable. This could be dependent on the property characteristics of the facility, how soon the escape is detected, what and when food is set out, etc. If the facility property contains wooded or other areas that may provide suitable shelter that is not heavily used by people, the animal may not go far immediately. To increase the chances of keeping an escaped wolf (when visual contact has been lost) within the facility's perimeter fence and catching the animal, food should be provided at strategic locations, e.g., outside the pen where the animal escaped and at undisturbed places on the facility property, especially near natural runways such as old roads, paths, etc., as soon as possible. The food and the area around the food should be checked morning and evening for evidence of the presence of animals, including tracks. If a wolf leaves the facility property, it will likely travel where cover is available, e.g., along wooded streams, old roadways, path margins, etc. However, wolves will travel on roads with traffic.

3. If an animal leaves the facility's property and is not captured within 24 hours, the RWSSP coordinator will contact FWS Red Wolf Program personnel and accompany them to the site to assist with the capture.

4. The facility should have all necessary equipment available, except for leg-hold traps. Equipment should include 3-4 functional wolf nets, catch poles, transfer crates, cap-chur or telinject equipment (gun, jab stick, drugs, etc.), current county road maps, hand-held radios and appropriate personnel available to handle any situation at any hour. If some of this equipment is not available, the RWSSP coordinator and FWS should be so advised, otherwise it will be assumed that the facility has sufficient equipment. Additionally, determining the availability of food in the form of road kills through

appropriate local agencies, e.g., State Wildlife or Transportation Department, etc., may prove helpful.

5. There is no reason to involve the media if it is determined that the animal is still on the facility's property or if the animal is captured within 24 hours. If it is determined that the animal has left the property and has not been captured within 24 hours, the public is owed the truth, i.e., an animal has escaped, we are attempting to capture it, and here are instructions to the public regarding their observations of the animal, (including a phone number to call and instructions for them not to attempt capturing the animal). However, in all media contacts, efforts should be made to allay the public's fear to the degree possible. For example, inform them that the animal is shy and afraid of humans and that it is therefore not likely to be a danger. However, if cornered, it might respond aggressively to humans, and it might injure or kill pets for food or in defense.

6. A follow-up report from the RWSSP representative should be sent to the RWSSP coordinator. This report should address important points such as how the escape occurred, what corrective measures will be taken, when the escape was detected, was food provided and when the coordinator was contacted, news media interactions and response (attach newspaper articles), was the animal recaptured, when was it recaptured and how, was FWS called in and when, etc. The purpose of this report is not to direct blame but to be used to evaluate these procedures and to minimize the likelihood of subsequent escapes and to maximize response and efficiency in future escapes.

SECTION 4

Transport and Shipping

4.1 Vehicle Transport. For local transport to another pen, veterinary clinic, etc., the crate should be placed in a well-ventilated vehicle. Under no circumstances should a crated wolf be left for any period of time in direct sunlight or in areas where there is excessive noise and commotion. Adult wolves that have experience being crated will normally lie quietly in a crate while being transported. Young wolves that have not been crated may thrash around or periodically bite at the crate. This reaction can be reduced by loosely covering the crate with a light tarpaulin.

When transporting red wolves in vehicles over long distances, animals should be transported in secured crates similar to those used for air transport. Equipment such as nets, noose, medical kit, cellular phone should be taken along in the event that problems arise. The vehicle should be well ventilated and the temperature monitored so that the wolves do not become over-heated or chilled. At no time should wolves be transported over long distances in the back of a pick-up truck. The maximum amount of time to consider transporting wolves over long distances should not exceed twenty-four hours. Red wolves should never be crated the night before a scheduled transport (vehicle or air). If departures are anticipated when it is dark, the wolf should be moved to a smaller holding area to allow for easier crating.

4.2 Air Transport. Red wolves should not be sedated for shipment, and should be fasted 12 hours prior to shipment. Water should be available up until the animal is crated for shipment. Considerations when arranging animal shipments include length of time the animal is confined to the shipping crate and the amount of extra handling required by the airlines. Federal and International Air Transport Association (IATA) regulations will sufficiently address the welfare of the animal; however, animal facility personnel should still monitor the shipment and do everything possible to coordinate with the airlines. Whenever possible, non-stop flights should be scheduled between the shipping points. Few airlines will have direct flights to where animals need to be shipped or flights in which a change of planes is not required. Most shipments have a change of planes, which means a layover time for the animal. Most airlines require at least a two-hour layover to guarantee transfer to the new plane. Layover times can vary from the minimum two hours to six hours or more. For this reason, personnel making arrangements should always try to find the airline with the shortest total flight time including layover time. Some airlines will try to make an earlier transfer if they have more than one flight per day to the end destination. If at all possible, inter-airline

transfers should be avoided because they require greater coordination and increase the risk of the animal missing its flight.

Another factor to consider is the time of arrival at the new facility. Most facilities have personnel that will pick up arrivals at any time. These arrangements must be confirmed with the receiving facility before shipping times can be confirmed with the airline, in case there is a conflict at the receiving facility.

(a) Shippers Responsibilities. The airline will require that a reservation to ship the animal be made several days in advance. Once the shipping arrangements have been confirmed, the shipper, as a precaution, should contact each airline freight manager that will be involved in the shipment by phone to inform the freight manager on the nature of the animal and any special precautions such as avoiding heat by keeping the animals cage in a well ventilated shaded area during periods that it is on the ground. All airlines have specific time requirements for receiving animals for shipment. Most require the animal to be on location at least two hours in advance. During certain times of the year, as before Christmas, they may change that requirement to three or more hours before the scheduled departure. Shippers must always be alert to these changes, and the fact that sometimes airline personnel may make a mistake and give out wrong information. A call to the airline, an hour before leaving for the airport, is advisable to confirm times and check for cancellations due to technical problems or weather conditions. Carriers will periodically place a ban on animal shipments through certain locations if the temperature is considered too risky. These bans are beyond our control and have delayed many shipments by days or weeks. The sending facility should contact the receiving facility to confirm that the shipment has been made and to inform them of any changes.

(b) Receivers Responsibilities. The receiver should call the airlines to confirm that the animal made its connection, if the plane will be arriving on schedule, or if the animal was loaded on an earlier arriving flight. If the intended connection were not made, other arrangements would have to be made; e.g. catching a later flight on the same airline or changing to another airline that would be arriving sooner. Most airlines require at least half an hour to unload and process an incoming animal. After an animal has arrived and been transported to the receiving facility, the receiving facility should call the shipper to inform them that the animal arrived safely.

Because of the amount of shipping that takes place in the RWSSP, facilities that are shipping wolves can usually count on receiving a crate from another facility. However, this may not occur equitably if a facility is shipping multiple wolves out and only receiving one in return. Therefore, if a facility requests that their crates be returned, please honor that request by returning their crate.

Because of the number of wolves involved in the RWSSP and other factors such as genetic and demographic considerations, reintroduction needs, reproductive studies, births and deaths, number of facilities, etc., determining wolf shipments is an involved process. Cooperating RWSSP facilities are to be acknowledged for their unselfish participation in this program and are to be commended for their understanding in these matters.

(c) Required Documents. Paperwork required to accompany the animal include but may not be limited to: two copies of a health certificate from the shippers veterinarian; two copies of a USDA animal transfer form; and animal health records with the animal(s) studbook and transponder number. State permits may also be needed where required. Animal data transfer forms, available through the American Association of Zookeepers, should also be included. Labels for the outside of the crate showing the shippers name and address, the receivers name and address and any additional instructions should be attached to the crate.

4.3 Shipping Preparation. Recently, strict adherence by many airline carries to IATA policies and in some cases outright refusal to ship a wolf has compounded shipping difficulties. Sue Behrns will coordinate with facility representatives about the availability of acceptable crates. Acceptable crates will vary according to airlines, the location (city) of the airline and even airline personnel accepting a wolf for shipment. Sue has modified standard 500 size Vari Kennels by having aluminum frames fabricated for the windows. PVC coated (14 gauge) ½ x ½ in. (1.25 x 1.25 cm) welded wire is cut to size and sandwiched between the aluminum frame and the inside of the window, then secured to the crate with pop rivets. The inside of the doors are covered with the same gauge wire and attached with "J" clips or hog rings. After the animal is in the crate, the door is wired to the crate by way of pre-drilled holes through the plastic entrance. Burlap is fitted over the doors and windows to reduce stress on the animals during shipment. Time required to modify each crate for shipment is ~ 2 hours. Sue can be contacted for details.

In the past, wood slated bottoms that were screwed to the bottom of the crate were used in the shipment of red wolves to allow urine and feces drop away from the animal during shipment. This procedure has been stopped because some wolves will chew on and splinter the wood increasing the chance of injury during shipment. All that is required is an appropriate amount of straw or similar material as bedding.

Water and/or food containers are not to be placed in the crate for shipping. Wolves can and will chew the small plastic dishes supplied with the crates. Some airlines or individual airline personnel may argue the need for this based on

USDA guidelines and/or their own airline policies. If the shipping facility has had difficulty with particular airlines strictly enforcing this policy refer to USDA Subpart F, Section 3.139 (c), "A sufficient quantity of food and water shall accompany the live animal to provide food and water for such animals for a period of at least 24 hours, except as directed by hibernation, veterinary treatment, normal fasts, and other professionally accepted practices." A letter written by the facility's veterinarian stating this may help alleviate some delays or refusal to ship. If transport exceeds 24 hours, an attendant from the shipping facility must accompany the wolf.

SECTION 5

Behavioral and Social Characteristics

5.1 Introduction. Annual summaries of the current free-ranging population support the social nature of red wolves and evidence that the relationship of paired wolves demonstrate solid pair bonding. The number and composition of a family group can vary according to their location, available habitat and food, and time of year. Age of dispersal can vary with 18 months of age being the average age for young wolves to disperse from the family group. This type of social structure is difficult to maintain in the captive setting. Inherent limitations in pen space dimensions and the need to relocate designated wolves to other facilities supersedes strict adherence to long term red wolf social groupings. Therefore, a number of scenarios are possible with regard to the number, age, sex and relationship of red wolves that would occupy an enclosure.

5.2 Separating Wolves. The effect of removing wolves, e.g. for transfer, from an enclosure to achieve program objectives can be difficult to quantify. Separating an adult pair or 1-2 year old offspring from adults or siblings from siblings have occurred since the red wolf program began with anecdotal observations showing substantial variability in the reaction that an individual wolf may have. In human terms, reactions by wolves seem to vary from possible short-term distress to indifference. Adult pairs that have been together for several years and have been separated per breeding recommendations and have bred and produced pups with their former pen mate visible in an adjacent enclosure. There have also been occasions at Graham where a single neonate has been removed from its sire and dam and successfully introduced to another adult pair with pups so that the single pup would derive the benefits of growing up with littermates.

5.3 Animal Introductions. Red wolf introductions can generally be characterized as non-events, however interactions should be monitored for the first few days to get a general idea of compatibility. There have been a few reports of a resident wolf reacting to a newly introduced wolf as though it were intruding on an established territory. This can be particularly evident in the case of wolves that were born and remain at a facility. There have been several reports of females in this scenario dominating an introduced male, thereby minimizing the chances for successful breeding. The degree to which established wolves will dominate introductions may be minimized by holding the resident (established) animal in an adjacent holding area and allowing the newly introduced wolf access to the pen while having visual contact with its new mate or by removing the resident wolf and allowing the new wolf a few days to become familiar with its new surroundings.

(See section 1.2 for requirements for holding wolves in adjacent holding areas). If holding pens are not available, using a "howdy" type of introduction can be tried by crating the resident and new wolf in separate crates and allowing each wolf to see and smell each other for about 30 minutes before releasing both in the enclosure.

SECTION 6

Reproduction

6.1 Introduction. Red wolves are monestrus with breeding occurring from February through March. Gestation is 60-63 days. The average litter size is 4 - 5 with the earliest births to date occurring on April 4; the latest litter produced was May 25. Red wolves paired as close to the breeding season as February have resulted in successful breeding/whelping.

6.2 Physical Characteristics. The male wolf's genitals recede in the spring and are quite reduced through the summer. The genitals will begin to develop in the late fall, as indicated by distinct enlargement of the scrotum and general hair loss from the scrotal area. The breeding status of the female is not as easily detected. Vaginal discharge and swelling of the vulva may be observed during estrus. It should be understood that excessive vaginal discharge during the breeding season, continued spotting after the season, spotting shortly after whelping, or spotting at any other time of the year may indicate that the female has a uterine or bladder infection. As with other canids, uterine infections can be fatal but can be effectively treated if detected early.

It is likely that the dynamics of the family group acts to suppress reproduction from young wolves remaining with their parents. Most healthy female wolves may appear to undergo estrus during their first breeding season at about nine months of age, which in some cases may be a false estrus. Some captive born female wolves have successfully bred during their first season, when removed from their parents and paired with a breeding age male. However, female red wolves are generally not reproductively viable until their second season at approximately 21 months of age. Captive born males have successfully bred during their second breeding season at approximately 21 months of age, when removed from their parents and paired with a breeding age female. While, viable sperm has been collected from one year olds, volume is generally low.

6.3 Behavioral Characteristics. Occasionally, determination of a female's breeding status can be aided by noting changes in the behavior of the male toward the female, the female's acceptance of the male, or by observing copulation (See Appendix C). Although some females may show obvious physical signs of pregnancy, as with other canids, most may not appear pregnant until just prior to whelping. False pregnancies do occur. Noting loss of belly fur is generally a good indication that the female may be close to whelping.

6.4 Parental Care. In general, adult red wolves provide excellent parental care. They may whelp inside the den provided by a facility or in a place of their own choosing. The females will usually run from the den when someone approaches. If the female stays with her pups she may still be in the process of whelping. If this occurs, leave her alone and come back in 3 to 4 hours. After this period, if the female does not leave the den, noose her and check the pups, taking care that she does not jump around and inadvertently injure one of the pups. Normally, the female will lie still while the pups are examined. If straw or similar material is used as a bedding substrate, the material should be built-up beneath the pups whenever they are checked. Even with this intervention, adults will provide for the litter though she may move them to another location in the pen. The female will not kill the pups because humans touched them. Females normally will not allow the male access to the den when occupied by pups.

Average red wolf pups weights between 255g – 350g. Between days ten and fourteen eyes should open and by about four weeks the pups may begin to wander out of the den, staying near the den entrance. By about six weeks the pups will spend more time outside the den. Depending on the female's level of tolerance, pups are weaned at about eight to nine weeks.

6.5 Hand Rearing. In most cases, hand rearing will not be an option for red wolf pups. This approach to husbandry may be emotionally difficult for animal keepers who recognize that more could be done to aid the survival of individual pups. However, it must understood that the objective of the program is not to simply produce red wolves, but to produce wolves that are representative of their species and behaviorally suitable for potential reestablishment in the wild. Therefore, hand rearing or other extraordinary measures to increase survival rates will not be used unless such care is absolutely necessary for the survival of the species. Such intervention will be evaluated on a case by case basis through direct consultation with the SSP coordinator and program advisors.

6.6 Contraception. Where separation of wolves during breeding season is not possible, methods recommended by the AZA Contraception Advisory Group will be used. Progestin contraceptives are currently recommended for use in red wolves. Long term use of progestins in domestic dogs has been associated with undesirable side effects, such as an increased incidence of mammary tumors and infections of the uterus. Because of the similarity between domestic dog and wolf reproduction, progestin contraceptives could also increase the incidence of these diseases in red wolves.

Melegestrol Acetate Subcutaneous Implants (MGA). MGA is currently being used in red wolves when separation is not possible and is available through

Dr. Ed Plotka. Short-term use may be acceptable (less than 2 years). However, it should be understood that there might be an increased risk of developing uterine infections and mammary gland tumors in wolves on these contraceptives. Also, this contraceptive method requires surgical procedures to insert and remove the implant. The RWSSP coordinator will contact facilities regarding use of contraceptive methods for designated individuals.

SECTION 7

Nutrition

7.1 Introduction. Periodically, red wolves from the captive population may be selected for release into the wild. However, it is not the responsibility of the RWSSP to feed red wolves a diet that they would find in the wild. Biologists in the field will assist the wolves in making the transition from a captive diet to natural prey items. Consistency in types of food is important to ensure that the wolves will readily eat when being transferred from one facility to another.

7.2 Captive Diet. Feeding requirements of red wolves have generally not been a problem in the RWSSP, as long as a good quality commercial (dry) dog food is provided. Because of the number of commercial foods made, their availability, and cost it is difficult to recommend a specific brand. Wolves maintained in Tacoma have done well on food with label guarantees ranging from 22 – 28% protein, 8 – 18% fat, and 2 – 4% fiber. Vitamin supplements for red wolves are normally not required. Adding commercial carnivore log to dry chow may be needed to encourage some wolves to eat, although should not be the primary component of their diet.

7.3 Feeding Regime. Individual feeding containers should be available for each wolf housed at a facility and placed a good distance apart within the pen to minimize one wolf monopolizing the food. Stainless steel pans or bowls work well because they are durable and rust proof. Food containers should be cleaned and disinfected daily. Some facilities report effective use of community feeders. Regardless of the feeding method used, individuals should be monitored to insure that each wolf is getting enough food or that an individual is getting too much food.

(a) Adults. Each adult wolf should be fed amounts based on weight, age and activity level in accordance with manufactures recommended amounts. The wolves should be fasted one day per week except when there are young and during sustained cold weather, ~ 40° F (4.4° C). In some cases, it may be necessary to feed near dusk to prevent avian scavengers from eating the wolves' food. It is not necessary to change to a higher protein food during breeding and whelping season as long as a good quality food is being provided. The female's food intake should be monitored during this period and additional food provided if warranted.

Stool quality can vary from wolf to wolf and may change due to activity level, stress, parasites, infection, bowel disease, food allergy, or changes in food.

Stool quality should be monitored daily with general appearance compared to the gastrointestinal grading sheet described in Appendix D. Grades 2 – 4 are not uncommon in healthy red wolves with Grade 3 considered typical. Changes in an individual wolf's stool quality should be related to the veterinarian and chronic diarrhea should be reported to the RWSSP veterinary advisor.

(b) Pups. Feeding puppy or growth chow is not required for red wolf pups, besides the adults will eat this food. When pups are seen eating food provided to the adults, food amounts can be increased. More feed pans can also be added if needed. Pups should also have access to water by placing a shallow pan of water near the adult water source.

7.4 Bones. Bones may be fed on a random basis. These bones must be very large (beef/horse variety). The wolves should be monitored as gastrointestinal problems may occur with some individuals. Bones are good enrichment items and do seem to increase activity.

7.5 Whole Food. Providing food other than dry chow can occur on a random basis. However, whole food, e.g., rabbit, mice, deer, etc., should not be the primary diet as this can make switching to dry chow more difficult when a wolf is moved to another facility that may not have the ability to offer similar food items. When feeding whole food, it is important that the source is reputable and that food items are not contaminated.

SECTION 8

Health and Medical Care

8.1 Introduction. This section offers general veterinary guidelines to RWSSP cooperators and is based on experience and input from RWSSP, FWS, and program advisors. As with other animals in the collection, red wolf health should be evaluated visually on a routine basis and via annual physical examination.

8.2 Quarantine. A thirty (30) day quarantine period is appropriate unless otherwise directed by the institution's veterinarian. Keepers caring for the resident wolves, mustelids, viverrids and other canids should not care for red wolves in quarantine. Disinfectants such as 0.5% sodium hypochlorite (bleach) or chlorhexidine diocetate (Nolvasan® Aveco, Fort Dodge, IA 50501) should be used in footbaths and to disinfect equipment and food dishes.

A complete physical examination should be performed during the quarantine period. This should include: 1) a general physical exam, 2) an ophthalmological exam, 3) a hematological exam (complete blood count, serum chemical analysis), 4) analysis for the presence of *Dirofilaria immitis* antibodies and circulating microfilaria, 5) storage of serum for serological screening for antibodies to common canine pathogens, 6) fecal exam (flotation and direct); 7) dental examination, 8) vaccinations, and 9) permanent identification with a subcutaneous implantation of a transponder or verify that existing transponder is in place and can be read. At the completion of the quarantine period, when the wolves are determined to be in good health, they can be introduced to the resident collection.

8.3 Preventative Medicine. Preventative medicine programs will vary per institution because of the variety of geographic locations in which captive red wolves are located, associated differences in climate, and varying prevalence of diseases and common parasites.

(a) Adults. Previous medical and nutritional records including past immunizations should be reviewed and complete medical records should be kept during quarantine and continued for the life of the animal.

1. Parasite Control. Red wolves should be tested for heartworm antibody and microfilaria via occult and difil heartworm tests. In areas where heartworm infestations are a problem, wolves with a negative test result should receive ivermectin (Ivomec, Merck & Co., Rahway, NJ 07065) at 3-5 µg/kg body weight) orally every 30 days.

Stool samples should be inspected at least twice a year for ova and parasites and treated accordingly.

- Roundworms - fenbendazole (Panacure® American Hoescht, Somerville, NJ 08876) 50 mg/kg Per Os, once per day x 3days (for Strongyloides use the same dose for 5 days) **or** pyrantel pamoate (Strongid T® Pfizer, Inc., NY, NY 10017) **or** ivermectin (MSD-AgVet Division of Merck & Co., Inc. Rahway, NJ 07065) 200 µg/kg IM or PO.
- Tapeworms - Praziquantel (Droncit®, Haver-Lockhart, Shawnee, KS 66201), see package insert for graded doses.
- Coccidia – sulfadimethoxine (Albon® Pfizer, Exton, PA 19341). 50 mg/kg PO once daily for first day then 25 mg/kg once daily for 14-21 days.

2. Vaccinations. Adults should be vaccinated annually. Do not use modified live virus vaccines of **canine** cell origin. The following vaccinations are recommended:

- Canine Distemper - Galaxy D® (Solvay, Mendota Heights, MN 55120) modified live mammalian cell origin.
- Parvo virus - Vanguard CPV®, (Smith Kline Beecham, Exton, PA) killed **or** Parvocine® (Biocor Inc., Omaha, NE 68134) killed.
- Leptospirosis - Leptoferm 5-way® (Smith Kline Beecham, Exton, PA) recommended in endemic areas or in the face of an outbreak: should vaccinate every 6 months.
- Corona virus - Duramune® CV-K (Fort Dodge Labs Inc., Iowa 50501) killed; if recommended for your area.

AND

- Rabies - Imrab® (Rhone Merieux, Athens, GA 30601). Killed vaccine.

(b) Pups. Red wolf pups should be examined thoroughly by the staff veterinarian the day they are discovered. Pups should be examined for numbers, sexes, general physical condition and presence of congenital defects (cleft palate, imperforate anus, umbilical problems, heart defects, clear lungs). The female will usually run from the den when someone enters the pen. If she stays with her pups she may still be in the process of whelping. If this is the case leave her alone and come back in three to four hours. If you have a female wolf that is finished whelping and will not leave the den, she can be carefully noosed and the pups checked while she is restrained. The normal response of the females will be to lay still. On only a

few occasions has a female required noosing and in those cases she laid still while the pups were examined. Male pup testicles' should be checked when one of the last series of vaccinations is given to confirm that the testicles have descended. Testicles should be descended by six months of age or sooner.

1. Parasite Control. Begin deworming the pups orally at 10 to 14 days of age with pyrantel pamoate (Strongid-T®, Pfizer Inc., New York, NY 10017) at 5 mg/kg. Continue deworming once every two weeks up to 10 weeks of age. Deworm pups in conjunction with scheduled vaccinations.

2. Vaccination. Pups should be vaccinated on the following schedule:

   - Canine Distemper – Galaxy D® at 8, 10, 12, 14 and 16 weeks.
   - Parvo virus - Vanguard CPV® **or** Parvocine® at 8, 10, 12, 14, 16, 18 and 20 weeks.
   - Rabies - Imrab® at 16 weeks.

8.4 Ectoparasites. Flea, tick or mite problems will vary with location and climate. Some red wolves will also vary in their sensitivity to flea bites. As with domestic dogs, flea control may require comprehensive management programs involving area sprays or foggers such as Siphotrol® (Vet Kem - division of Sandoz, Dallas, TX 75234) **or** Duratrol® (3M Animal Care Products, St. Paul, MN 55144). Topical insecticides (carbaryl powder, pyrethrin dips and sprays) and systemic treatments (Program® Ciba Animal Health, Greensboro, NC 27419) may also be needed to supplement premise management programs. If a nursing mother or her pups have a significant flea problem, carbaryl powder is the treatment of choice. After dusting the dam, make sure to use a moist cloth to wipe down the teat line. Carbaryl is safe to use on neonates.

At a number of facilities, the incidence of fly bites to the ears of wolves prompted the development of a survey by Yvonne Strode and Dawn Fleuchaus, Racine Zoo to evaluate insect problems and methods of control (Table 1 – 4).

8.5 Medical Problems. The following conditions are some of the medical problems that have been encountered in the captive red wolf population.

(a) Ulcerative Pododermatitis. This condition is seen in red wolf neonates and is noted by abrasions of the footpads and abdomen on pups. Initially, the pup may appear bright and alert but at a later stage is found to be depressed and

lethargic; in this case an indication of sepsis. It is suggested that neonates locomote within the den box pushing aside bedding (e.g., straw, shavings) thereby abrading their soft footpads and abdominal skin on the rough den box floor. These abrasions are then infected with the skin's normal flora (*Staphylococcus ssp.*). From there the infection can spread into the blood stream leading to septicemia. Footpad lesions may not show up until a couple of days after birth, therefore continuous early monitoring is important. This condition has been minimized by the addition of a substantial layer of dirt to the den box prior to whelping. When the pups begin to spend more time out of the den the dirt can be removed. Treatment of the infection has been successful with the use of injectable and oral amoxacillin, clavulanate/amoxicillin, or lincomycin (Jones, pers. comm., 1995). The skin lesions should be cleaned with dilute chlorhexidene solution and treated with topical antibiotics.

(b) Uterine Infections. On several occasions, investigation of vaginal spotting or discharge soon after whelping or unrelated to parturition resulted in diagnosis of metritis. Treatment with antibiotics suggested by culture and sensitivity led to resolution of the infections.

(c) Malocclusions. Throughout the pup's vaccination series, their bite should be examined for evidence of underbite. This has been observed in a number of red wolves to date. Not all members of a litter will have this condition. Call the RWSSP coordinator if you find any pups with this condition.

(d) Hepatic (Portal) Shunt. This has been diagnosed in two red wolves. While at this time it is not considered a major problem in the population, it is something to be monitored in red wolves. Any congenital abnormalities should be reported to the RWSSP Veterinary Advisor.

(e) Stomach Torsions. On occasion, as with large breeds of dogs, stomach torsions have been encountered. Animals with this condition may decline rapidly and should be handled as a medical emergency.

(f) Progressive Retinal Atrophy (PRA). PRA is a disease seen in domestic dogs in which the retinal cells gradually die until the animal is completely blind. This may occur over the course of months or years. The early sign of this disease is blindness at night or in dimming light. This can be hard to detect in red wolves, as they are often not closely observed at night. Other signs include unusually dilated pupils and an increase in reflectivity of the eyes (eyeshine). Eventually the wolf will demonstrate signs of blindness. Because PRA is an inherited disease there is a notable concern for the red wolf breeding program. Red wolves carrying only one of the recessive genes for PRA will not become blind. Yet if two such animals breed and pass on their recessive genes, the offspring will manifest the disease. In an effort to

monitor the spread of this disease, animals demonstrating vision problems and retinal lesions should be reported to the RWSSP Coordinator or Veterinary Advisor.

(g) Inflammatory Bowel Disease (IFBD). The increasing reports of chronic diarrhea, active gastrointestinal disease, and thickened bowel on physical exam or necropsy have stimulated many questions concerning the cause of these problems in red wolves. Pathologists have suggested that the IFBD is due to chronic antigenic stimulation, i.e. parasitism, food allergy, chronic infection. When dealing with these findings diagnostics should include serum analysis in the fasted animal for canine TLI, cobalamine and folate (in addition to CBC and chemistry) to further clarify the etiology. Similar findings should be reported to the RWSSP Veterinary Advisor.

8.6 Physiological Norms. Normal clinical pathology values (Table 5) and physiological normal values (Table 6).

8.7 Chemical Immobilization. Red wolves should be manually restrained and immobilized via hand injection or crated and taken to the hospital and immobilized there. In virtually all cases, the use of pole syringe, blowgun, telinject, or power projection system (powder charge, CO_2, compressed air) is not required for captive red wolves. Drug and dosage recommendations (Table 7).

8.8 Euthanasia. When it is determined that a red wolf's health is compromised enough to consider euthanasia, the institutions veterinarian and red wolf representative should contact the RWSSP coordinator, in addition to any other specialist, to discuss the situation. These decisions will be made on a case by case basis and always with the welfare of the individual wolf and overall objectives of the program in mind. In emergency cases, the RWSSP's institutional representative and veterinarian may together determine what is best for the animal in question.

8.9 Necropsy Protocol. The necropsy protocol and tissue/sample check list developed by Dr. Linda Munson, University of California at Davis and adopted by the Canid TAG will remain in effect (Appendix F). Although tissue samples are no longer being sent to Dr. Munson, a copy of the necropsy and pathology reports must be sent to the RWSSP Coordinator. One aspect of the protocol relates to the skull. Do not remove brain tissue unless your veterinarian feels that clinical signs of (CNS) central nervous system/brain disease were a factor in the death (or need to euthanize) a wolf. Determining the cause of death (and therefore removing brain tissue if necessary) supersedes the need for having an intact skull for preservation.

The RWSSP Coordinator will inform facilities about the disposition of skulls as directed by the Red Wolf Recovery Coordinator. Facilities that want to prepare a skull or skin for use in educational programs should inform the RWSSP Coordinator. Written approval from the Red Wolf Recovery Coordinator would then be requested and all materials would remain the property of the Service and could be recalled at their discretion. All other remains should be disposed of according to each institution's policy.

SECTION 9

Research

9.1 Introduction. From time to time, RWSSP cooperators may be interested in conducting research or may receive proposals from outside their institution to conduct red wolf related research. Depending on the type of research being proposed and the source of the request, a formal proposal may be requested for review and approval by USFWS project personnel and/or the RWSSP Management Group. When a formal research proposal is requested, the following policy developed by the Red Wolf Recovery Coordinator will be observed:

The Red Wolf Program receives numerous requests for cooperation and collaboration with researchers. These requests range from providing general information to extensive involvement in cooperatively implementing research studies. Probably the most common request is for us to provide specimen material, such as blood samples.

As a general policy we should cooperate with all requests that can be accommodated without causing any significant impact on our primary objective of recovering the red wolf. As a public agency we should not selectively choose researchers to cooperate with unless there are good reasons; e.g., we are funding research with a particular researcher and do not want to compromise that research until completed; the researcher is under investigation, has admitted guilt, or has been convicted of violation(s) of wildlife statutes; etc. Questionable requests should be referred to the Red Wolf Recovery Coordinator for a decision.

We must be sure to inform the researchers that material provided from any endangered red wolf will remain the property of the U. S. Fish and Wildlife Service (Service). The researchers, in effect, operate as subpermittees under the Service. Sample material remaining after research is completed must be returned to the Service or be made available to other researchers when instructed to do so by the Service.

We need to advise the researchers that we would like to have the opportunity to review the results, reports, and/or publications generated from research prior to their publication. Authorship of publications would, of course, be the prerogative of the principle investigator. However, we should certainly accept coauthorship if it is offered, and would hope that significant contributions would be so rewarded.

A copy of this memo should be provided to researchers as documentation that they have been advised of this policy. Service files on the project should document the fact and date that this policy memo was provided to the researcher.

TABLE 1: Severity of problem caused by insects to red wolves at responding institutions.

INSTITUTION	RESPONDENT/PHONE #	SPECIFIC PROBLEM(S)	SEVERITY
Alligator River National Wildlife Refuge	Jonathon Windley/919-473-2557	flies around pens	slight
		fleas and ticks inside pens & on animals	slight-moderate
Beardsley Zoological Gardens	Dan Goff/203-576-7535	flies biting ears (affects some more than others)	moderate-severe
Brevard Zoo	Shawn Heflick/407-254-9453	flies biting ears (only 1 of 3 affected)	moderate
Burnet Park Zoo	Tom LaBarge/315-468-0607	flies biting ears	slight
Chaffee Zoological Park	Lisa Peach/209-498-4692	fleas (only 1 of 2 affected)	severe
Chattanooga Nature Center	Otis Allen/423-821-1160	no problems (have only had wolves one month)	
Fossil Rim Wildlife Center	Mary Jo Stearns/817-897-2960	flies on ears	moderate
		fleas and ticks	moderate
Great Plains Zoo	Kurt Simon/605-367-7003	flies biting ears	severe
Great Smokey Mountains National Pk	Christopher Lucash/423-448-6835	no problems	
Gulf Island National Seashore	Gary Hopkins/601-875-9057	NA (wolves are free ranging)	
Henson Robinson Zoo	Catherine Short/217-753-6217	flies biting ears	moderate
Knoxville Zoological Gardens	Sherrie Hardin/423-637-5331 X362	mosquitos	moderate
		fleas (affected one female that was transferred)	
Miller Park Zoo	Jack Ritter/309-434-2250	fly bites to ears (only on males)	moderate-severe
Mill Mountain Zoo	Laurie Spangler/540-343-3241 X14	fleas seen on pups	no skin irritation
National Zoological Park	Belinda Reser/202-673-4809	no problems	
North Carolina Zoo	Sheryl L. Staaden/910-879-7600	no problems	
Oglebay Zoo	Marty Mendel/304-243-4029	no problems	
Pittsburgh Zoo	Lee Nesler/412-665-3651	flies biting/chewing ears	moderate
Point Defiance Zoo/Graham Facility	Susan Behrns/206-847-4833	flies biting ears (affects very young & older wolves)	moderate
Racine Zoo	Dawn Fleuchaus/414-636-9189	flies biting ears	severe
Roger William Park Zoo	Dave Wetzel/401-785-3510	no problems	
Ross Park Zoo	Deidre Flaherty/607-724-5461	no problems	
St.Vincent National Wildlife Refuge	Thomas E. Lewis/904-653-8808	no problems	
The Texas Zoo	Sherry K. Hornstein/512-573-7681	no problems	
The Wilds	Mark Jacobs/614-638-2220	flies biting ears	slight
Trevor Zoo	Dr. Tousignant/914-677-3704	no problems	
Wild Canid Survival & Research Center	Kim Bishop/314-938-6490	flies biting ears & backs (affects some more than others)	moderate
		ticks (younger animals affected)	severe

TABLE 2: Control methods used for flies.

INSTITUTION	PRODUCT NAME	MANUFACTURER	METHOD/FREQ OF APPLICATION	RESULTS	COMMENTS
Alligator River	Fly Trap	Sterling IPC	hang trap in area of fly source/replace every 3-4 months	excellent	simple,very effective
Beardsley Zoo	Repel-X		mix to proper dilution & mix with bedding or mulch/daily	good	helps but doesn't solve problem
	Ectrin Cattle Tage	Fermata Animal Health	place in ear and cut to 1/4 size/seasonally	poor	hard to keep tags in
	Solar Fly Traps	Arbico	set baited traps in problem areas	good	need to be maintained
	Revap E.C.	Fermata Animal Health	mix to proper dilution & spray non-animal areas/weekly	poor	may work better if applied more often
Brevard Zoo	Bronco-equine fly spray	Farnam	impregnated fire hose strips placed in mouth of night house/spray strips biweekly	?	method just instituted
Burnet Park Zoo	Fly wipe	various	sprayed directly on animal/daily	poor	could not get close; worried about eyes; worked well on Arctic wolves
	garlic		1 clove/diet/day	fair (?)	
Chaffee Zoo	crushed garlic		in food daily	good	worked on grey wolves, not needed on reds
Fossil Rim	Permectin II		direct spray on animals as opportunity arises		
			direct spray on grass & in houses/monthly	good	
Great Plains Zoo	VIP Fly Repellent Oint.	Pet Chemicals	smeared on ground & wolves roll bodies in it (use on alternate days with Tick Killer)	fair	some wolves roll more others;have to use a lot
	Adam's Tick Killer	SmithKlineBeekham	smeared on ground & wolves roll bodies in it	no change	can spray timber wolf when he walks up to fence, not red wolves
	Defend EXspot	Pitman-Moore	1cc between shoulder blades, 1cc at base of tail & a couple drops on each ear/monthly	?	method just implemented
Miller Park Zoo	Defend		1cc tube-1/2 dribbled on each ear; other 1cc tube dribbled down middle of back/bi-weekly	no change	just went from monthly to bi-weekly appl.
	Trap-N-Toss fly traps		hang 3-5 around perimeter of 150'X150' exhibit/ replace when full-usually 1-2 weeks	?	traps catch lots of flies
	Golden Malrin Fly Bait		hung in refillable dispersers on exterior of exhibit/ refilled every 4-6 weeks	?	use quite a bit of product
	Fly Parasite	Beneficial Insectary	parasitic pupae spread around exhibit/weekly from spring till fall in 1995	?	
Pittsburgh Zoo	Swat		applied directly to ears/whe animal is down	good	too few opportunities to apply
	fly spray		sprayed around head avoiding eyes/daily	fair	difficult to apply short duration of protection

TABLE 2 (cont): Control methods used for flies.

INSTITUTION	PRODUCT NAME	MANUFACTURER	METHOD/FREQ OF APPLICATION	RESULTS	COMMENTS
Point Defiance Zoo	Schreiners Healing Liniment	Restoration Products	applied by hand to both ears & top of head/twice a day	excellent	used in severe cases only
	Rescue disposable fly traps	Sterling International	hang around perimeter fence & in between pens/ change when needed	excellent	still see an animal with a problem
Racine Zoo	Insectrin X	Hess & Clarke Inc	sprayed directly on animals from pump sprayer/ when flys are covering ears	good	
	Permectrin Dust	Anchor	sprinkled in wooden shelters & on ground where animals lay/weekly	good	also added to straw that is given as enrichment
	Permethrin LPS	Dionne	40cc/gal water-spray outside perimeter in tall grass & bushes/twice a week (repeat after rain)	fair	
	Vectro System Fly Zapper	Micro-gen	electrical bug zapper in keeper area of building/ sticky board replaced as needed	excellent	building is free of flies
	Fly Terminator	Farnam	placed on ground around perimeter fence-baited per directions + one dead fish/change as necessary	good	
Trevor Zoo	Fly Pest Strips		hang in key areas of zoo/changed monthly		
Wild Canid Center	Permethrin spray	various	fine mist sprayed around facility perimeter/weekly	excellent	County Health Dept applies
	Permethrin spray	various	sprayed on ground in certain areas of enclosures to initiate scent rolling/as needed	fair	wolves stop rolling when they get used to scent

TABLE 3: Control methods used for fleas and ticks.

PRODUCT NAME	METHOD/FREQ OF APPLICATION	RESULTS	INSTITUTION	COMMENTS
Yard and Kennel Spray; Vet-Kem	sprayed in pens with hose end sprayer/as needed	excellent	Alligator River	
Spot-ON; Miles Inc	applied directly to backline at .8 cc per 10bs/as needed	excellent	Alligator River	used for severe cases
Program (Luferuron); Ciba-Geigy	oral during feeding/monthly year round	excellent	Chaffee Zoo	
	orally during feeding/monthly	prevention	North Carolina Zoo	discontinued-could not separate wolves easily
	orally during feeding/monthly during flea season	excellent	Racine Zoo	
	orally during feeding/monthly	good	The Texas Zoo	
Basis; Ciba Geigy	sprayed yard at beginning of flea season	excellent	Chaffee Zoo	no longer needed
Ivomectin	orally in food - 1 ml per 110lbs/one time does	fair (?)	Fossil Rim	used for severe cases
Ovitrol Plus; Vet-Kem	wrapped pups in lightly sprayed towels/when pups are handled for other reasons	fair	Mill Mountain Zoo	no skin irritation seen
Permethrin Spray	sprayed from high powered hose into ground to penetrate ground & leaf litter around perimeter of enclosures/beginning & end of season	excellent	Wild Canid	County Health Dept applies

TABLE 4: Exhibit/management modifications used to reduce the problems with insects.

MODIFICATION	COMMENTS	INSTITUTION
Keep grass between exhibits down.		Beardsley Zoo
No standing water.		
Exhibit mowed once a week.	Cuts down on insect densities	Brevard Zoo
Drain standing water.		Burnet Park Zoo
Natural dens and low vegetation.		Chaffee Zoo
Keep vegetation low.		Great Plains Zoo
Minimal vegetation, dens partially below gound & disinfected regularly, allow shallow holes to be dug.		Knoxville Zoo
Mow area when too tall, no standing water, added more shelters.		Miller Park Zoo
Minimal Vegetation, allow holes to be dug.		Mill Mountain Zoo
Drain standing water by filling in holes in yard.		Pittsburgh Zoo
Occasionally trim vegetation		The Wilds
Natural dens.	Seems to be a natural fly control.	Trevor Zoo
Keep vegetation mowed.	Extremely helpful in tick control.	Wild Canid
Do not allow leaf litter to accumulate	Eliminated tick cover.	
Remove standing water.	Extremely helpful in fly control.	
Keep compost heaps & garbage containers covered.		

Clinical Pathology Records Report - ISIS/In-House Reference Values

Scientific name: *CANIS RUFUS*
Common Name: RED WOLF

		ISIS Values			Pt. Defiance Zoo & Aquarium In-House Values				
		Mean	S.D.	(N)	Mean	S.D.	Min.	Max.	(N)
WBC	*10^3/UL	11.48	2.802	(52)	11.85	3.86	5.500	24.20	(132)
RBC	*10^6/UL	7.20	0.79	(51)	6.362	1.091	1.610	8.220	(132)
HGB	GM/DL	18.1	1.6	(50)	16.05	2.36	8.900	20.90	(132)
HCT	%	54.6	5.0	(57)	47.17	7.19	27.70	60.90	(132)
MCH	*10^3/UL	25.3	1.4	(50)	25.03	2.56	0.030	33.54	(133)
MCHC	uug	33.1	1.4	(49)	34.06	0.80	32.13	36.08	(133)
MCV	fL	76.7	4.2	(50)	73.46	7.33	0.080	96.89	(133)
SEGS	*10^3/UL	7.677	2.392	(52)	8647	2980	3432	17415	(130)
BANDS	*10^3/UL	0.113	0.021	(2)	202.0	138.0	0.000	310.0	(4)
LYMPHOCYTES	*10^3/UL	2.221	1.132	(52)	1496	777	242.0	3660	(130)
MONOCYTES	*10^3/UL	0.511	0.344	(46)	853.8	405.5	85.00	2000	(130)
EOSINOPHILS	*10^3/UL	1.038	0.751	(52)	631.0	454.9	0.000	1972	(130)
BASOPHILS	*10^3/UL	0.098	0.060	(8)	16.65	26.95	0.000	98.00	(81)
NRBC	/100 WBC	1	1	(9)	2.389	1.420	1.000	6.000	(18)
PLATE. CNT.	*10^3/UL	253	57	(34)	299.6	84.4	138.0	575.0	(128)
RETICS	%				23.50	19.66	9.600	37.40	(2)
GLUCOSE	MG/DL	125	23	(54)	116.2	18.9	63.00	167.0	(120)
BUN	MG/DL	23	7	(53)	21.06	5.95	10.00	42.00	(119)
CREAT.	MG/DL	1.2	0.3	(52)	1.245	0.240	0.500	1.900	(119)
URIC ACID	MG/DL	0.4	0.3	(25)					
CA	MG/DL	9.8	0.6	(53)	8.991	0.466	7.700	9.900	(122)
PHOS	MG/DL	3.7	0.8	(48)	3.524	0.934	1.200	6.000	(123)
NA	MEQ/L	150	5	(49)	147.7	2.6	142.0	155.0	(122)
K	MEQ/L	4.8	0.5	(49)	5.038	0.397	4.100	6.200	(122)
CL	MEQ/L	115	4	(42)	115.7	2.6	106.0	124.0	(122)
IRON	MCG/DL	136	84	(13)					
CHOL	MG/DL	126	48	(40)	148.0	40.6	74.00	336.0	(122)
TRIG	MG/DL	40	22	(30)					
T.PROT. (C)	GM/DL	6.5	0.5	(53)	5.829	0.793	3.500	7.200	(69)
T.PROT. (R)	GM/DL				6.196	0.520	4.800	7.000	(52)
ALBUMIN (C)	GM/DL	3.4	0.2	(26)	3.148	0.580	1.400	4.200	(122)
GLOBULIN (C)	GM/DL	3.2	0.4	(26)	2.835	0.512	1.900	4.200	(123)
AST (SGOT)	IU/L	60	26	(43)	52.78	25.62	24.00	186.0	(120)
ALT (SGPT)	IU/L	66	23	(46)	56.73	22.48	20.00	144.0	(120)
T. BILI.	MG/DL	0.2	0.1	(42)	0.219	0.084	0.100	0.500	(121)
D. BILI	MG/DL	0.0	0.0	(10)					
I. BILI.	MG/DL	0.2	0.1	(10)					
AMYLASE	U/L	584	260	(19)	445.3	158.5	245.0	1198	(68)
ALK.PHOS.	IU/L	43	20	(52)	28.88	15.72	2.000	70.00	(121)
LDH	IU/L	232	176	(26)					
CPK	IU/L	292	160	(15)	265.8	338.5	74.00	2443	(69)
OSMOLARITY	MOSMOL/L				289.0	0.0	289.0	289.0	(1)

Clinical Pathology Records Report - ISIS/In-House Reference Values

Scientific name: *CANIS RUFUS*
Common Name: RED WOLF

		ISIS Values Mean	S.D.	(N)	Pt. Defiance Zoo & Aquarium In-House Values Mean	S.D.	Min.	Max.	(N)
A/G RATIO	GM/DL				1.146	0.324	0.500	2.000	(123)
AG	MG/DL				13.28	5.04	6.000	29.00	(122)
Body Temperature:		38.9	1.0	(19)					
CT4					0.951	0.397	0.100	2.100	(63)
CO2	MMOL/L	19.9	3.4	(13)	18.76	4.05	6.000	26.00	(122)
ESR	MM/HR				1.684	1.572	0.000	8.000	(57)
GGT	IU/L	3	3	(9)	3.613	1.606	0.000	8.000	(119)
HI	MG/DL				33.07	28.81	4.000	127.0	(121)
ICT					0.000	0.000	0.000	0.000	(73)
LIPASE	U/L	280	104	(6)	193.8	79.9	72.00	375.0	(71)
LI	MG/DL				6.375	4.647	0.000	20.00	(120)
MCH	PG				25.60	4.78	22.10	78.00	(132)
MCHC	%				34.06	0.80	31.90	36.20	(132)
MCV	FML				73.73	2.55	67.00	82.00	(131)
OSMOLALITY	MOSMOL/KG				298.5	6.3	281.0	316.0	(123)
PROGESTERONE	NG/DL	36.90	0.000	(1)	8.990	11.29	1.010	16.97	(2)
RDW	K/UL				14.84	1.11	12.50	17.70	(130)
SP RAT					29.23	2.40	23.00	35.00	(60)
TETRAIODOTHY	UG/DL				4.656	10.26	0.700	32.00	(9)

Table 6. Red wolf physiological norms

	Normal Resting	Anesthetized with ketamine/xylazine or Telazol
Temperature	99 – 103° F	Same
Heart Rate	49 – 59 bpm	80 – 105 bpm
Respiration Rate	15 – 20 rpm	12 – 25 rpm

Table 7. Red Wolf Immobilization

Weight	Range: 45 – 80 lbs (20 – 36 kg) Average female weight: 52 lbs. (24 kg) Average male weight: 62 lbs. (28 kg)
Recommended Drug	10.0 mg/kg ketamine plus 2.0 mg/kg xylazine IM, antagonize w/ 0.1 mg/kg yohimbine (divided ½ IM, ½ IV)
Supplemental Drug	5.0 mg/kg ketamine IM
Alternative Drugs	6.0 – 13.0 mg/kg Telazol IM 10.0 mg/kg ketamine plus 1.0 mg/kg promazine IM
Comments	If using xylazine, wait at least 45 minutes after last ketamine injection before administering yohimbine
Respiratory Stimulant	2.0 mg/kg Doxapram HCL (Dopram) IV
Adjuvant	0.04 mg/kg atropine IM

Appendix A

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Appendix A

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Appendix C

Management Ethogram

General *Canis* behavior is well documented. It has been suggested by numerous researchers that closely-related canids (such as the *Canis* spp.)exhibit similar forms of behavior, though the frequency of the behaviors exhibited may differ significantly. This ethogram is not comprehensive; instead it is intended that captive managers use the following as a descriptive guide to red wolf behavior to assist them in day-to-day management. The behaviors listed below are grouped by general category. It should be noted, however, that many of these behaviors may be observed in more than one context.

General Behavior

Approach	Wolf approaches another within 3 body lengths. An interaction is not required. Distinguished from threat by lack of an aggressive body posture or vocalizations.
Follow	Wolf follows another. They do not have to be within 3 body lengths, but actions must be simultaneous, i.e. both are locomoting, one behind the other.
Pass	Wolves locomote towards each other and pass (one going one direction, one the other) without stopping or interacting.

Elimination Behavior

Leg lift urination	Urination with one leg lifted off the ground
Squat urination	Urination from a squatting position
Over mark	Wolf urinating or defecating almost immediately over the urination or defecation of another
Scent rolling	Rub of head, neck, back on a surface; often where another urinated or defecated. May include repeated full rolls in the area.
Defecate	Wolf discharges fecal material
Scrape mark	Wolf uses both front and back paws to tear at the ground (dirt is usually thrown up in the process). May follow the discharge of urine.

Social Behavior

Play	Interactive behavioral event identified by play initiator (see below) performed by one wolf at some point in the interaction (most often at the beginning, but does not have to be). Play initiator can include any of the following: a) play bow-crouches on forelegs with elevated rear-end and straightened rear legs, b) exaggerated approach-gait is bouncy, or a rush; head and shoulders frequently moved side to side; c) approach/withdrawal-can be at different speeds or showing physical intent to move away (e.g. rock back and forth); d) general movements such as head tosses, paw raises, etc. Play types include prone play, one up/one down play, wrestle play, locomotor play and ambush/stalk play. Wolves can show any of the following: play grin", head up and alert, tail wag, mouth open with lips drawn back and tongue out.
Unreciprocated play	Wolf directs any play type towards another, who subsequently does not respond, actively tries to avoid the initiator, or becomes aggressive towards it.
Self play	Wolf chasing its own tail, limb biting, etc.
Sniff or Lick A-G	Sniff or lick another's ano-genital area
Present A-G	Female stands or walks with hindquarters oriented to male's face, back slightly arched, base of tail deflected up or to the side.
Attempted mount	Male attempts to mount female, though mount is unsuccessful (may be from an incorrect orientation)
Mount	Wolf standing behind another resting upon its back with forepaws clasped around the midsection/pelvic region; may be followed by pelvic thrusts
Copulatory tie	Male and female are joined in a 'mount' position that lasts a minimum of 60 sec. Seen after several short pelvic thrusts followed by 2 or 3 deep thrusts resulting in the tie. Back-to-back ties are sometimes seen.

Agonistic Behavior

Charge	Wolf lcomotes rapidly towards another exhibiting one or more of the following behaviors: ears back, head down, hair is piloerect, forelegs are stiff. Recipient may either react (see fight, passive/active submission) or retreat.

Threat	Orientation towards another with threat facial expressions (vertical retraction of the lips and baring of the front teeth), stiff legs, ears forward and erect, elevated tail and hair piloerected. May be accompanied by a growl.
Chase	Wolf runs after another (both are running).
Passive Submission	Wolf approaches another in crouch or semi-crouch position with body oriented sideways to partner; head typically rolled sideways while looking at partner. May be accompanied by whimper/whine and licking intentions towards partner.
Active Submission	In presence of another, wolf falls or lies on its side or back, often with hindlegs raised and ears back. May be accompanied by whimper/whine. Can follow passive submission.
Fight	An interaction which is usually initiated by a charge from one individual, followed by both growling, rising up or partially up on their hind legs and batting at each other with the forepaws or grasping each other around the neck or shoulders (sparring), growling, threatening and each attempting to pin the other to the ground.

Vocalization

Whimper/whine	Soft whine, usually emitted while approaching another in a submissive (ears back, somewhat crouched) position
Aggressive vocalization	Growl, bark
Distress vocalization	High pitched, whining or yelping vocalization. Often associated with submissive behavior

Grade 1

Greater than two-thirds of the feces in a defecation are liquid. The feces have lost all form, appearing as a puddle or squirt.

Grade 2

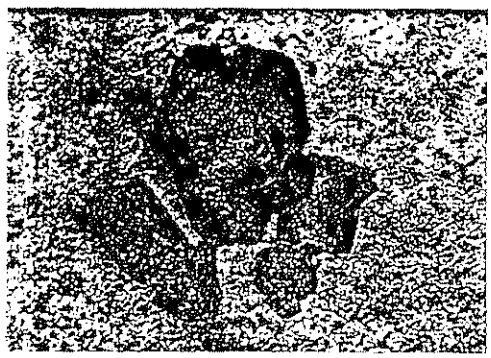
Soft-liquid feces are an intermediate between soft and liquid feces. Approximately equal amounts of feces in a defecation are soft and liquid.

Grade 3

Greater than two-thirds of the feces in a defecation are soft. The feces retain enough form to pile but have lost their firm cylindrical appearance.

Grade 4

Firm-soft feces are an intermediate between the grades of firm and soft. Approximately equal amounts of feces in a defecation are firm and soft.



Grade 5

Greater than two-thirds of the feces in a defecation are firm. They have a cylindrical shape with little flattening.

Appendix E

American Zoo and Aquarium Association
RED WOLF SSP NECROPSY PROTOCOL

INSTITUTION/OWNER_____

ADDRESS_____

_____

CANID SPECIES _____

CANID NAME OR ISIS #_____ STUD BOOK #_____ SEX _____

BIRTH DATE/AGE _____ WEIGHT _____

PRODUCED PUPS?_____ HOUSED WITH OPPOSITE SEX?_____

DATE OF DEATH _____ DATE OF NECROPSY _____

HISTORY: (briefly summarize clinical signs, circumstances of death):

Please have your pathologist perform histopathology on tissues. Then send the gross examination worksheets and pathologist's report to Will Waddell at Point Defiance Zoo.

Animal ID #_____

GROSS EXAMINATION WORKSHEET

PROSECTOR:_____

GENERAL CONDITION: (Nutritional condition, physical condition)
Neonates: examine for malformations (cleft palate, deformed limbs etc).

SKIN: (Including pinna, feet)

MUSCULOSKELETAL SYSTEM: (Bones, joints, muscles)

BODY CAVITIES: (Fat stores, abnormal fluids)
Neonates: assess hydration (tissue moistness)

HEMOLYMPHATIC: (Spleen, lymph nodes, thymus)

RESPIRATORY SYSTEM: (Nasal cavity, larynx, trachea, lungs, regional lymph nodes)
Neonates: determine if breathing occurred (do the lungs float in formalin?)

CARDIOVASCULAR SYSTEM: (Heart, pericardium, great vessels)

DIGESTIVE SYSTEM: (Mouth, teeth, esophagus, stomach, intestines, liver, pancreas, mesenteric lymph nodes). **Neonates:** is milk present in stomach?

Animal ID #_____

URINARY SYSTEM: (Kidneys, ureters, urinary bladder, urethra)

REPRODUCTIVE SYSTEM: (Testis/ovary, uterus, vagina, penis, prepuce, prostate, mammary glands, placenta)

ENDOCRINE SYSTEM: (Adrenals, thyroid, parathyroids, pituitary)

NERVOUS SYSTEM: (Brain, spinal cord, peripheral nerves)

SENSORY ORGANS (Eyes, ears)

PRELIMINARY DIAGNOSES:

LABORATORY STUDIES:(List bacterial and viral cultures submitted and results, if available)

Animal ID #_____

FIXED TISSUE CHECK LIST

Preserve the following tissues in 10 % buffered formalin at a ratio of 1 part tissue to 10 parts formalin. Tissues should be no thicker than 1 cm. INCLUDE SECTIONS OF ALL LESIONS AND SAMPLES OF ALL TISSUES ON THE SSP REQUIRED TISSUE LIST.

SSP REQUIRED TISSUES and recommended tissue sampling procedures:

__**Salivary gland**
__**Oral/pharyngeal mucosa and tonsil** -plus any areas with erosions, ulcerations or proliferative lesions.
__**Tongue** - cross section near tip including both mucosal surfaces.
__**Lung** - sections from several lobes including a major bronchus
__**Trachea**
__**Thyroid/parathyroids** - leave intact.
__**Lymph nodes** - cervical, mediastinal, bronchial, mesenteric and lumbar. Cut transversely.
__**Thymus**
__**Heart** - longitudinal sections including atrium, ventricle and valves from right and left sides.
__**Liver** - sections from 3 lobes, including gall bladder
__**Spleen** - Cross sections including capsule.
__**GI Tract** - 3 cm long sections of:
Esophagus
Stomach - multiple sections from cardia, fundus (body), and antrum of pylorus
Small intestines - duodenum, jejunum, ileum
Large intestines - cecum, colon
__**Omentum** - ~3 cm square
__**Pancreas** - representative sections from two areas including central ducts
__**Adrenal** - entire gland with transverse incision.
__**Kidney** -cortex and medulla from each kidney
__**Urinary bladder, ureters, urethra** - cross section of bladder and 2 cm sections of ureter and
__**Reproductive tract** - Entire uterus and ovaries with longitudinal cuts into lumens of uterine horns. Both testes (transversely cut) with epididymis. Entire prostate, transversely cut.
__**Eye** - both eyes intact. Remove extraocular muscles and periorbital tissues.
__**Brain** - cut longitudinally along midline. Submit entire brain (retain small part of frontal cortex frozen if desired) and **pituitary gland.**
__**Spinal cord** (if neurologic disease) - sections from cervical, thoracic and lumbar cord.
__**Diaphragm and Skeletal muscle** - cross section of thigh muscles
__**Opened rib or longitudinally sectioned 1/2 femur** - marrow must be exposed for proper fixation
__**Skin** - full thickness of abdominal skin, lip and ear pinna.

FROZEN TISSUE: Store in plastic bags at -70 or -20 C for toxicology: Liver, brain, kidney and (if possible) antemortem serum and plasma frozen. If you suspect an infectious disease, also

freeze samples of small intestines, lung, spleen, and lymph nodes.

NEONATAL NECROPSY PROTOCOL

Please follow the adult protocol in addition to the following:

1. Check umbilical stump and surrounding tissues
2. Check for malformations (cleft palate, deformed limbs, heart defects)
3. Assess hydration (tissue moistness) and evidence of nursing (milk in stomach).
4. Determine if breathing occurred (do the lungs float in formalin?)
5. Check foot pads for erosions and ulcers

ADDITIONAL TISSUES FOR HISTOPATHOLOGY FROM NEONATES:

All tissues from the adult necropsy check-list.

Umbilicus (including external and internal vessels and surrounding skin.

Foot pads from all feet.

Extra sections of lung.